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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 37/24, 37/36, 39/00, C07K 3/00, 13/00, 15/00, 17/00, C07H 17/00, C12Q 1/00, G01N 33/53, C12N 5/00, 1/20, 15/00

A1

(11) International Publication Number: WO 95/01801

(43) International Publication Date: 19 January 1995 (19.01.95)

(21) International Application Number: PCT/US94/07762

(22) International Filing Date: 8 July 1994 (08.07.94)

(30) Priority Data:
08/089,300 9 July 1993 (09.07.93) US

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(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

**Published**
*With international search report.*

(54) Title: GROWTH DIFFERENTIATION FACTOR-6

(57) Abstract

Growth differentiation factor-6 (GDF-6) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-6 polypeptide and polynucleotide sequences.

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# GROWTH DIFFERENTIATION FACTOR-6

## BACKGROUND OF THE INVENTION

### 1. *Field of the Invention*

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF-$\beta$) superfamily, which is denoted, growth differentiation factor-6 (GDF-6).

### 2. *Description of Related Art*

The transforming growth factor $\beta$ (TGF-$\beta$) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al., Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al., Cell*, 51:861-867, 1987), the activins (Mason, *et al., Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos (Thomsen, *et al., Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al., J. Biol. Chem.*, 265:13198, 1990). The TGF-$\beta$s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF-$\beta$ family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. Studies have shown that when the pro-region of a member of the TGF-$\beta$ family is coexpressed with a mature region of another member of the TGF-$\beta$ family, intracellular dimerization and secretion of biologically active homodimers occur (Gray, A., and Maston, A., *Science*, 247:1328, 1990). Additional studies by Hammonds, *et al.*, (*Molec. Endocrin.* 5:149, 1991) showed that the use of the BMP-2 pro-region combined with the BMP-4 mature region led to dramatically improved expression of mature BMP-4. For most of the family members that have been studied, the homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al., Nature*, 321:779, 1986) and the TGF-$\beta$s (Cheifetz, *et al., Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function.

## SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-6, a polynucleotide sequence which encodes the factor, and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell proliferative disorders, especially those involving placental tissue.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of placental origin and which is associated with GDF-6. In another embodiment, the invention provides a method for treating a cell proliferative disorder by suppressing or enhancing GDF-6 activity.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows expression of GDF-6 mRNA in placenta. The arrow denotes the position of the major mRNA species..

FIGURE 2 shows nucleotide and predicted amino acid sequence of murine GDF-6. The putative pentabasic processing site is boxed.

FIGURE 3 shows the alignment of the C-terminal sequences of GDF-6 with other members of the TGF-$\beta$ superfamily. The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize alignment.

FIGURE 4 shows amino acid homologies among different members of the TGF-$\beta$ superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-6 and a polynucleotide sequence encoding GDF-6. GDF-6 is expressed in placental tissue. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of placental origin which is associated with GDF-6 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder by using an agent which suppresses or enhances GDF-6 activity.

The TGF-$\beta$ superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-6 protein of this invention and the members of the TGF-$\beta$ family, indicates that GDF-6 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-6 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

The expression of GDF-6 in the placenta suggests a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to pregnancy and cell proliferative diseases. Abnormally low levels of the factor may be indicative of impaired function in the placenta while abnormally high levels may be indicative of hypertrophy or hyperplasia. Hence, GDF-6 may be useful in detecting primary and metastic neoplasms of placental origin. In addition, GDF-6 may also be useful as an indicator of developmental anomalies in prenatal screening procedures.

Several members of the TGF-$\beta$ superfamily possess activities suggesting possible applications for the treatment of cell proliferative disorders, such as cancer. In particular, TGF-$\beta$ has been shown to be potent growth inhibitor for a variety of cell types (Massague, *Cell*, 49:437, 1987). MIS has been shown to inhibit the growth of human endometrial carcinoma tumors in nude mice (Donahoe, *et al., Ann. Surg.*, 194:472, 1981), and inhibin $\alpha$ has been shown to suppress the development of tumors both in the ovary and in the testis (Matzuk, *et al., Nature*, 360:313, 1992) GDF-6 may have a simlar actiity and may therefore be useful as an anti-proliferative agent, such as for the treatment choriocarcinoma.

Many of the members of the TGF-$\beta$ family are also important mediators of tissue repair. TGF-$\beta$ has been shown to have marked effects on teh formation of collagen and causes of striking angiogenic response in teh newborn mouse (roberts, *et al., Proc. Natl. acad. Sci., USA*, 83:4167, 1986). The BMP's can induce new bone growth and are effective for the treatment of fractures and other skeletal defects (Glowacki, *et al., Lancet*, 1:959, 1981; Ferguson, *et al., Clin. Orthoped. Relat. Res.,*, 227:265, 1988; Johnson, *et al., Clin Orthoped. Relat. Res.*, 230:257, 1988). GDF-6 may have simlar activities and may be useful in repair of tissue injury caused by trauma or burns for example.

GDF-6 may play a role in the regulation of uterine function during pregmancy, and therefore, GDF-6, anti-GDF-6 antibodies, or antisense polynucleotides may be useful in preventing premature labor.

The term "substantially pure" as used herein refers to GDF-6 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-6 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity

of the GDF-6 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-6 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-7 remains. Smaller peptides containing the biological activity of GDF-7 are included in the invention.

The invention provides polynucleotides encoding the GDF-6 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-6. It is understood that all polynucleotides encoding all or a portion of GDF-6 are also included herein, as long as they encode a polypeptide with GDF-6 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-6 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-6 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-6 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a genomic DNA sequence containing a portion of the GDF-6 gene. The sequence contains an open reading frame corresponding to the predicted C-terminal region of the GDF-6 precursor protein. The encoded polypeptide is predicted to contain a potential pentabasic proteolytic processing site. Cleavage of the precursor at this site would generate a mature biologically active C-terminal fragment of 120 amino acids with a predicted molecular weight of approximately 13,600.

The C-terminal region of GDF-6 following the putative proteolytic processing site shows significant homology to the known members of the TGF-$\beta$ superfamily. The GDF-6 sequence contains most of the residues that are

highly conserved in other family members (see Figure 3). Among the known family members, GDF-6 is most homologous to BMP-2 (57% sequence identity) (see Figure 4).

Minor modifications of the recombinant GDF-6 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-6 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-6 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-6 biological activity.

The nucleotide sequence encoding the GDF-6 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-6 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al., Nucl. Acid Res.*, 9:879, 1981).

The development of specific DNA sequences encoding GDF-6 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al., Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-6 peptides having at least one epitope, using antibodies specific for GDF-6. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-6 cDNA.

DNA sequences encoding GDF-6 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the GDF-6 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-6 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-6 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal region of GDF-6 is expressed from a cDNA clone containing the entire coding sequence of GDF-6. Alternatively, the C-terminal portion of GDF-6 can be expressed as a fusion protein with the pro- region of another member of the TGF-$\beta$ family or co-expressed with another pro- region (see for example, Hammonds, *et al., Molec. Endocrin.* 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-6 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect

or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

The invention includes antibodies immunoreactive with GDF-6 polypeptide or functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, *et al.*, *Nature*, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well as fragments thereof, such as Fab and $F(ab')_2$, which are capable of binding an epitopic determinant on GDF-6.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. Malignant cells (i.e. cancer) develop as a result of a multistep process. The GDF-6 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, cells in placental tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-6 could be considered susceptible to treatment with a GDF-6 suppressing reagent. One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of placental tissue which comprises contacting an anti-GDF-6 antibody with a cell suspected of having a GDF-6 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-6 is labeled with a compound which allows detection of binding to GDF-6. For purposes of the invention, an antibody specific for GDF-6 polypeptide may be used to detect the level of GDF-6 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is placental tissue. The level of GDF-6 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-6-associated cell proliferative disorder. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immuno-assays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene,

polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific anti-hapten antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for in vivo diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are $^{111}In$, $^{97}Ru$, $^{67}Ga$, $^{68}Ga$, $^{72}As$, $^{89}Zr$, and $^{201}Tl$.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include $^{157}Gd$, $^{55}Mn$, $^{162}Dy$, $^{52}Cr$, and $^{56}Fe$.

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-6-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-6-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-6-associated disease in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-6, nucleic acid sequences that interfere with GDF-6 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-6 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target GDF-6-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by GDF-6 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-6 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-6 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-6 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the GDF-6 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to Ψ2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag, pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-6 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 $\mu$m can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al., Trends Biochem. Sci.*, 6:77, 1981). In

addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al., Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which

contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-6 in placental tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to this tissue. Such applications include treatment of cell proliferative disorders involving this tissue. In addition, GDF-6 may be useful in various gene therapy procedures.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

## EXAMPLE 1

## IDENTIFICATION AND ISOLATION OF A NOVEL TGF-β FAMILY MEMBER

To identify a new member of the TGF-β superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family members: one region spanning the two tryptophan residues conserved in all family members except MIS and the other region

spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on mouse genomic DNA followed by subcloning the PCR products using restriction sites placed at the 5' ends of the primers, picking individual *E. coli* colonies carrying these subcloned inserts, and using a combination of random sequencing and hybridization analysis to eliminate known members of the superfamily.

GDF-6 was identified from a mixture of PCR products obtained with the primers
SJL141: 5'-CCGGAATTCGGITGG(G/C/A)A(G/A/T/C)(A/G)A(T/C)TGG(A/G)TI (A/G)TI(T/G)CICC-3' (SEQ ID NO:1)
SJL145:5'-CCGGAATTC(G/A)CAI(G/C)C(G/A)CAIG(C/A)(G/A/T/C)TCIACI(G/A) (T/C)CAT-3' (SEQ ID NO:2)

PCR using these primers was carried out with 2 $\mu$g mouse genomic DNA at 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min for 40 cycles.

PCR products of approximately 280 bp were gel-purified, digested with Eco RI, gel-purified again, and subcloned in the Bluescript vector (Stratagene, San Diego, CA). Bacterial colonies carrying individual subclones were picked into 96 well microtiter plates, and multiple replicas were prepared by plating the cells onto nitrocellulose. The replicate filters were hybridized to probes representing known members of the family, and DNA was prepared from non-hybridizing colonies for sequence analysis.

The primer combination of SJL141 and SJL145, encoding the amino acid sequences GW(H/Q/N/K/D/E)(D/N)W(V/I/M)(V/I/M)(A/S)P (SEQ ID NO:3) and M(V/I/M/T/A)V(D/E)(A/S)C(G/A)C (SEQ ID NO:4) respectively, yielded four previously identified sequences (BMP-4, inhibin $\beta$B, GDF-3 and GDF-5) and two novel sequences, which were designated GDF-6 and GDF-7 among 134 subclones analyzed.

## EXAMPLE 2

## EXPRESSION PATTERN AND SEQUENCE OF GDF-6

To determine the expression pattern of GDF-6, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. RNA isolation and Northern analysis were carried out as described previously (Lee, S.-J., *Mol. Endocrinol.*, 4:1034, 1990) except that hybridization was carried out in 5X SSPE, 10% dextran sulfate, 50% formamide, 1% SDS, 200 μg/ml salmon DNA, and 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. Five micrograms of twice poly A-selected RNA were electrophoresed on formaldehyde gels, blotted, and probed with GDF-6. As shown in Figure 1, the GDF-6 probe detected a single mRNA species expressed in placentas during late gestation.

To obtain a larger segment of the GDF-6 gene, a mouse genomic library was screened with a probe derived from the GDF-6 PCR product. The partial sequence of a GDF-6 genomic clone is shown in Figure 2a. The sequence contains an open reading frame corresponding to the predicted C-terminal region of the GDF-6 precursor protein. The predicted GDF-6 sequence contains a potential proteolytic processing site, which is boxed. Cleavage of the precursor at this site would generate a mature C-terminal fragment 120 amino acids in length with a predicted molecular weight of 13,600.

The C-terminal region of GDF-6 following the putative proteolytic processing site shows significant homology to the known members of the TGF-β superfamily (Figure 3). Figure 3 shows the alignment of the C-terminal sequences of GDF-6 with the corresponding regions of human GDF-1 (Lee, *Proc. Natl. Acad. Sci. USA*, 88:4250-4254, 1991), human BMP-2 and 4 (Wozney, *et al., Science,* 242:1528-1534, 1988), human Vgr-1 (Celeste, *et al., Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human OP-1 (Ozkaynak, *et*

*al., EMBO J.*, 9:2085-2093, 1990), human BMP-5 (Celeste, *et al., Proc. Natl. Acad. Sci. USA*, 87:9843-9847, 1990), human BMP-3 (Wozney, *et al., Science*, 242:1528-1534, 1988), human MIS (Cate, *et al., Cell*, 45:685-698, 1986), human inhibin alpha, βA, and βB (Mason, *et al., Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), human TGF-β1 (Derynck, *et al., Nature*, 316:701-705, 1985), humanTGF-β2 (deMartin, *et al., EMBO J.*, 6:3673-3677, 1987), and human TGF-β3 (ten Dijke, *et al., Proc. Natl. Acad. Sci. USA*, 85:4715-4719, 1988). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

GDF-6 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing.

FIGURE 4 shows the amino acid homologies among the different members of the TGF-β superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups. In this region, GDF-6 is most homologous to BMP-2 (57% sequence identity).

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

## SUMMARY OF SEQUENCES

SEQ ID NO: 1 is the nucleotide sequence for the GDF-6 primer, SJL141.

SEQ ID NO: 2 is the nucleotide sequence for the GDF-6 primer, SJL145.

SEQ ID NO: 3 is the amino acid sequence for the primer, SJL141.

SEQ ID NO: 4 is the amino acid sequence for primer SJL145.

SEQ ID NO: 5 is the nucleotide and deduced amino acid sequence for GDF-6.

SEQ ID NO: 6 is the deduced amino acid sequence for GDF-6.

SEQ ID NO: 7 is the amino acid for the C-terminal sequence of GDF-6.

SEQ ID NO: 8 is the amino acid for the C-terminal sequence of GDF-1.

SEQ ID NO: 9 is the amino acid for the C-terminal sequence of BMP-2.

SEQ ID NO: 10 is the amino acid for the C-terminal sequence of BMP-4.

SEQ ID NO: 11 is the amino acid for the C-terminal sequence of Vgr-1.

SEQ ID NO: 12 is the amino acid for the C-terminal sequence of OP-1.

SEQ ID NO: 13 is the amino acid for the C-terminal sequence of BMP-5.

SEQ ID NO: 14 is the amino acid for the C-terminal sequence of BMP-3.

SEQ ID NO: 15 is the amino acid for the C-terminal sequence of MIS.

SEQ ID NO: 16 is the amino acid for the C-terminal sequence of Inhibin-alpha.

SEQ ID NO: 17 is the amino acid for the C-terminal sequence of Inhibin-beta-alpha.

SEQ ID NO: 18 is the amino acid for the C-terminal sequence of Inhibin-beta-beta.

SEQ ID NO: 19 is the amino acid for the C-terminal sequence of TGF-beta-1.

SEQ ID NO: 20 is the amino acid for the C-terminal sequence of TGF-beta-2.

SEQ ID NO: 21 is the amino acid for the C-terminal sequence of TGF-beta-3.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

(ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-6

(iii) NUMBER OF SEQUENCES: 21

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Spensley Horn Jubas & Lubitz
(B) STREET: 1880 Century Park East, Suite 500
(C) CITY: Los Angeles
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 90067

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT
(B) FILING DATE: 08-JUL-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: TUMARKIN, LISA A., PH.D.
(B) REGISTRATION NUMBER: P-38,347
(C) REFERENCE/DOCKET NUMBER: FD2349

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: (619) 455-5110

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: SJL141

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..35
(D) OTHER INFORMATION: /note= "V=guanine, cytosine or adenine; N=adenine, cytosine, guanine or thymine; R=adenine or guanine; Y=cytosine or thymine; K=thymine or guanine; B=inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGGAATTCG GBTGGVANRA YTGGRTBRTB KCBGC

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: SJL145

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..29
(D) OTHER INFORMATION: /note= "R=adenine or guanine; S=cytosine or guanine; M=adenine or cytosine; N=adenine, cytosine, guanine or thymine; Y=cytosine or thymine; B=inosine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCR CABSCRCABG MNTCBACBRY CAT 33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: SJL141

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /note= "His=His, Gln, Asn, Lys, Asp or Glu; Asp=Asp or Asn; Val=Val, Ile or Met; Ala=Ala or Ser"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Trp His Asp Trp Val Val Ala Pro
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: SJL145

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..8
(D) OTHER INFORMATION: /note= "Val, position 1=Val, Ile, Met, Thr or Ala; Asp=Asp or Glu; Ala=Ala or Ser; Gly=Gly or Ala"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Val Asp Ala Cys Gly Cys
1 5

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 530 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: GDF-6

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 126..527

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCCCTGCTTG TAGTGTTCAC CAGATCGCAG CGCAAGAACC TGTTCACTGA GATGCATGAG 60

CAGCTGGGCT CTGCAGAGGC TGCGGGAGCC GAGGGGTCAT GGCCAGCGCC GTCGGGCTCC 120

CAGAC GCC GGG TCT TGG CTG CCC TCG CCC GGC CGC CGG CGG CGA CGC 167
Ala Gly Ser Trp Leu Pro Ser Pro Gly Arg Arg Arg Arg Arg
1 5 10

ACC GCC TTC GCC AGC CGT CAC GGC AAG CGA CAT GGC AAG AAG TCC AGG 215
Thr Ala Phe Ala Ser Arg His Gly Lys Arg His Gly Lys Lys Ser Arg
15 20 25 30

```
CTG CGC TGC AGC AGA AAG CCT CTG CAC GTG AAT TTT AAG GAG TTA GGC      263
Leu Arg Cys Ser Arg Lys Pro Leu His Val Asn Phe Lys Glu Leu Gly
                35                  40                  45

TGG GAC GAC TGG ATT ATC GCG CCC CTA GAG TAC GAG GCC TAT CAC TGC      311
Trp Asp Asp Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Tyr His Cys
            50                  55                  60

GAG GGC GTG TGC GAC TTT CCG CTG CGC TCG CAC CTT GAG CCC ACT AAC      359
Glu Gly Val Cys Asp Phe Pro Leu Arg Ser His Leu Glu Pro Thr Asn
        65                  70                  75

CAT GCC ATC ATT CAG ACG CTG ATG AAC TCC ATG GAC CCG GGC TCC ACC      407
His Ala Ile Ile Gln Thr Leu Met Asn Ser Met Asp Pro Gly Ser Thr
    80                  85                  90

CCG CCT AGC TGC TGC GTT CCC ACC AAA CTG ACT CCC ATT AGC ATC CTG      455
Pro Pro Ser Cys Cys Val Pro Thr Lys Leu Thr Pro Ile Ser Ile Leu
95                  100                 105                 110

TAC ATC GAC GCG GGC AAT AAT GTA GTC TAC AAG CAG TAT GAG GAC ATG      503
Tyr Ile Asp Ala Gly Asn Asn Val Val Tyr Lys Gln Tyr Glu Asp Met
                115                 120                 125

GTG GTG GAG TCC TGC GGC TGT AGG TAG                                  530
Val Val Glu Ser Cys Gly Cys Arg
            130
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 134 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Ala Gly Ser Trp Leu Pro Ser Pro Gly Arg Arg Arg Arg Arg Thr Ala
 1               5                  10                  15

Phe Ala Ser Arg His Gly Lys Arg His Gly Lys Lys Ser Arg Leu Arg
            20                  25                  30
```

Cys Ser Arg Lys Pro Leu His Val Asn Phe Lys Glu Leu Gly Trp Asp
35 40 45

Asp Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Tyr His Cys Glu Gly
50 55 60

Val Cys Asp Phe Pro Leu Arg Ser His Leu Glu Pro Thr Asn His Ala
65 70 75 80

Ile Ile Gln Thr Leu Met Asn Ser Met Asp Pro Gly Ser Thr Pro Pro
85 90 95

Ser Cys Cys Val Pro Thr Lys Leu Thr Pro Ile Ser Ile Leu Tyr Ile
100 105 110

Asp Ala Gly Asn Asn Val Val Tyr Lys Gln Tyr Glu Asp Met Val Val
115 120 125

Glu Ser Cys Gly Cys Arg
130

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: GDF-6 (C-terminal)

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..119

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Phe Ala Ser Arg His Gly Lys Arg His Gly Lys Lys Ser Arg Leu
1 5 10 15

Arg Cys Ser Arg Lys Pro Leu His Val Asn Phe Lys Glu Leu Gly Trp
20 25 30

Asp Asp Trp Ile Ile Ala Pro Leu Glu Tyr Glu Ala Tyr His Cys Glu
35 40 45

Gly Val Cys Asp Phe Pro Leu Arg Ser His Leu Glu Pro Thr Asn His
50 55 60

Ala Ile Ile Gln Thr Leu Met Asn Ser Met Asp Pro Gly Ser Thr Pro
65 70 75 80

Pro Ser Cys Cys Val Pro Thr Lys Leu Thr Pro Ile Ser Ile Leu Tyr
85 90 95

Ile Asp Ala Gly Asn Asn Val Val Tyr Lys Gln Tyr Glu Asp Met Val
100 105 110

Val Glu Ser Cys Gly Cys Arg
115

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 123 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: GDF-1

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..123

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly
1 5 10 15

Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp
20 25 30

His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln
35 40 45

Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro
50 55 60

Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro
65 70 75 80

Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile
85 90 95

Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr
100 105 110

Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg
115 120

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: BMP-2

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Glu Lys Arg Gln Ala Lys His Lys Gln Arg Lys Arg Leu Lys Ser
1 5 10 15

Ser Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp
20 25 30

Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His
35 40 45

Gly Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His
50 55 60

Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys
65 70 75 80

Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu
85 90 95

Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val
100 105 110

Glu Gly Cys Gly Cys Arg
115

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: BMP-4

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys
1 5 10 15

Asn Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp
20 25 30

Asn Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His
35 40 45

Gly Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His
50 55 60

Ala Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys
65 70 75 80

Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu
85 90 95

Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val
100 105 110

Glu Gly Cys Gly Cys Arg
115

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Vgr-1

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..119

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser Arg Gly Ser Gly Ser Ser Asp Tyr Asn Gly Ser Glu Leu Lys Thr
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp
20 25 30

Gln Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp
35 40 45

Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
50 55 60

Ala Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr
85 90 95

Phe Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val
100 105 110

Val Arg Ala Cys Gly Cys His
115

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: OP-1

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..119

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser Asp Gln Arg Gln
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp
20 25 30

Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr Tyr Cys Glu
35 40 45

Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala Thr Asn His
50 55 60

Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Glu Thr Val Pro
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser Val Leu Tyr
85 90 95

Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val
100 105 110

Val Arg Ala Cys Gly Cys His
115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: BMP-5

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..119

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ser Arg Met Ser Ser Val Gly Asp Tyr Asn Thr Ser Glu Gln Lys Gln
1 5 10 15

Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp
20 25 30

Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp
35 40 45

Gly Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
50 55 60

Ala Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro
65 70 75 80

Lys Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr
85 90 95

Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val
100 105 110

Val Arg Ser Cys Gly Cys His
115

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 120 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: BMP-3

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..120

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Glu Gln Thr Leu Lys Lys Ala Arg Arg Lys Gln Trp Ile Glu Pro Arg
1 5 10 15

Asn Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp
20 25 30

Ser Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser
35 40 45

Gly Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His
50 55 60

Ala Thr Ile Gln Ser Ile Val Arg Ala Val Gly Val Val Pro Gly Ile
65 70 75 80

Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
85 90 95

Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
100 105 110

Thr Val Glu Ser Cys Ala Cys Arg
115 120

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: MIS

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..116

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Gly Pro Gly Arg Ala Gln Arg Ser Ala Gly Ala Thr Ala Ala Asp Gly
1 5 10 15

Pro Cys Ala Leu Arg Glu Leu Ser Val Asp Leu Arg Ala Glu Arg Ser
20 25 30

Val Leu Ile Pro Glu Thr Tyr Gln Ala Asn Asn Cys Gln Gly Val Cys
35 40 45

Gly Trp Pro Gln Ser Asp Arg Asn Pro Arg Tyr Gly Asn His Val Val
50 55 60

Leu Leu Leu Lys Met Gln Ala Arg Gly Ala Ala Leu Ala Arg Pro Pro
65 70 75 80

Cys Cys Val Pro Thr Ala Tyr Ala Gly Lys Leu Leu Ile Ser Leu Ser
85 90 95

Glu Glu Arg Ile Ser Ala His His Val Pro Asn Met Val Ala Thr Glu
100 105 110

Cys Gly Cys Arg
115

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Inhibin-alpha

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..122

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Leu Arg Leu Leu Gln Arg Pro Pro Glu Glu Pro Ala Ala His Ala
1 5 10 15

Asn Cys His Arg Val Ala Leu Asn Ile Ser Phe Gln Glu Leu Gly Trp
20 25 30

Glu Arg Trp Ile Val Tyr Pro Pro Ser Phe Ile Phe His Tyr Cys His
35 40 45

Gly Gly Cys Gly Leu His Ile Pro Pro Asn Leu Ser Leu Pro Val Pro
50 55 60

Gly Ala Pro Pro Thr Pro Ala Gln Pro Tyr Ser Leu Leu Pro Gly Ala
65 70 75 80

Gln Pro Cys Cys Ala Ala Leu Pro Gly Thr Met Arg Pro Leu His Val
85 90 95

Arg Thr Thr Ser Asp Gly Gly Tyr Ser Phe Lys Tyr Glu Thr Val Pro
100 105 110

Asn Leu Leu Thr Gln His Cys Ala Cys Ile
115 120

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Inhibin-beta-alpha

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..122

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Arg Arg Arg Arg Arg Gly Leu Glu Cys Asp Gly Lys Val Asn Ile
1 5 10 15

Cys Cys Lys Lys Gln Phe Phe Val Ser Phe Lys Asp Ile Gly Trp Asn
20 25 30

Asp Trp Ile Ile Ala Pro Ser Gly Tyr His Ala Asn Tyr Cys Glu Gly
35 40 45

Glu Cys Pro Ser His Ile Ala Gly Thr Ser Gly Ser Ser Leu Ser Phe
50 55 60

His Ser Thr Val Ile Asn His Tyr Arg Met Arg Gly His Ser Pro Phe
65 70 75 80

Ala Asn Leu Lys Ser Cys Cys Val Pro Thr Lys Leu Arg Pro Met Ser
85 90 95

Met Leu Tyr Tyr Asp Asp Gly Gln Asn Ile Ile Lys Lys Asp Ile Gln
100 105 110

Asn Met Ile Val Glu Glu Cys Gly Cys Ser
115 120

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 121 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Inhibin-beta-beta

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..121

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Arg Ile Arg Lys Arg Gly Leu Glu Cys Asp Gly Arg Thr Asn Leu
1 5 10 15

Cys Cys Arg Gln Gln Phe Phe Ile Asp Phe Arg Leu Ile Gly Trp Asn
20 25 30

Asp Trp Ile Ile Ala Pro Thr Gly Tyr Tyr Gly Asn Tyr Cys Glu Gly
35 40 45

Ser Cys Pro Ala Tyr Leu Ala Gly Val Pro Gly Ser Ala Ser Ser Phe
50 55 60

His Thr Ala Val Val Asn Gln Tyr Arg Met Arg Gly Leu Asn Pro Gly
65 70 75 80

Thr Val Asn Ser Cys Cys Ile Pro Thr Lys Leu Ser Thr Met Ser Met
85 90 95

Leu Tyr Phe Asp Asp Glu Tyr Asn Ile Val Lys Arg Asp Val Pro Asn
100 105 110

Met Ile Val Glu Glu Cys Gly Cys Ala
115 120

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: TGF-beta-1

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

His Arg Arg Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys
1 5 10 15

Asn Cys Cys Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly
20 25 30

Trp Lys Trp Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu
35 40 45

Gly Pro Cys Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val
50 55 60

Leu Ala Leu Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys
65 70 75 80

Cys Val Pro Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly
85 90 95

Arg Lys Pro Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys
100 105 110

Lys Cys Ser
115

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: TGF-beta-2

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Lys Lys Arg Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp
1 5 10 15

Asn Cys Cys Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly
20 25 30

Trp Lys Trp Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ala
35 40 45

Gly Ala Cys Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val
50 55 60

Leu Ser Leu Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys
65 70 75 80

Cys Val Ser Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly
85 90 95

Lys Thr Pro Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys
100 105 110

Lys Cys Ser
115

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 115 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: TGF-beta-3

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Lys Lys Arg Ala Leu Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu
1 5 10 15

Asn Cys Cys Val Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly
20 25 30

Trp Lys Trp Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser
35 40 45

Gly Pro Cys Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val
50 55 60

Leu Gly Leu Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys
65 70 75 80

Cys Val Pro Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly
85 90 95

Arg Thr Pro Lys Val Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys
100 105 110

Lys Cys Ser
115

## CLAIMS

1. Substantially pure growth differentiation factor-6 (GDF-6) and functional fragments thereof.

2. An isolated polynucleotide sequence encoding the GDF-6 polypeptide of claim 1.

3. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.

4. The polynucleotide of claim 3, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.

5. An expression vector including the polynucleotide of claim 2.

6. The vector of claim 5, wherein the vector is a plasmid.

7. The vector of claim 5, wherein the vector is a virus.

8. A host cell stably transformed with the vector of claim 5.

9. The host cell of claim 8, wherein the cell is prokaryotic.

10. The host cell of claim 8, wherein the cell is eukaryotic.

11. Antibodies reactive with the polypeptide of claim 1 or fragments thereof.

12. The antibodies of claim 11, wherein the antibodies are polyclonal.

13. The antibodies of claim 11, wherein the antibodies are monoclonal.

14. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 11 with a specimen of a subject suspected of having a GDF-6 associated disorder and detecting binding of the antibody.

15. The method of claim 14, wherein the cell is a placental cell.

16. The method of claim 14, wherein the detecting is *in vivo*.

17. The method of claim 16, wherein the antibody is detectably labeled.

18. The method of claim 17, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.

19. The method of claim 14, wherein the detection is *in vitro*.

20. The method of claim 19, wherein the antibody is detectably labeled.

21. The method of claim 20, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.

22. A method of treating a cell proliferative disorder associated with expression of GDF-6, comprising contacting the cells with a reagent which suppresses the GDF-6 activity.

23. The method of claim 22, wherein the reagent is an anti-GDF-6 antibody.

24. The method of claim 22, wherein the reagent is a GDF-6 antisense sequence.

25. The method of claim 22, wherein the cell is a placental cell.

26. The method of claim 22, wherein the reagent which suppresses GDF-6 activity is introduced to a cell using a vector.

27. The method of claim 26, wherein the vector is a colloidal dispersion system.

28. The method of claim 27, wherein the colloidal dispersion system is a liposome.

29. The method of claim 28, wherein the liposome is essentially target specific.

30. The method of claim 29, wherein the liposome is anatomically targeted.

31. The method of claim 30, wherein the liposome is mechanistically targeted.

32. The method of claim 31, wherein the mechanistic targeting is passive.

33. The method of claim 31, wherein the mechanistic targeting is active.

34. The method of claim 33, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.

35. The method of claim 34, wherein the protein moiety is an antibody.

36. The method of claim 35, wherein the vector is a virus.

37. The method of claim 36, wherein the virus is an RNA virus.

38. The method of claim 37, wherein the RNA virus is a retrovirus.

39. The method of claim 38, wherein the retrovirus is essentially target specific.

40. The method of claim 39, wherein the moiety for target specificity is encoded by a polynucleotide inserted into the retroviral genome.

41. The method of claim 40, wherein the moiety for target specificity is selected from the group consisting of a sugar, a glycolipid, and a protein.

42. The method of claim 41, wherein the protein is an antibody.

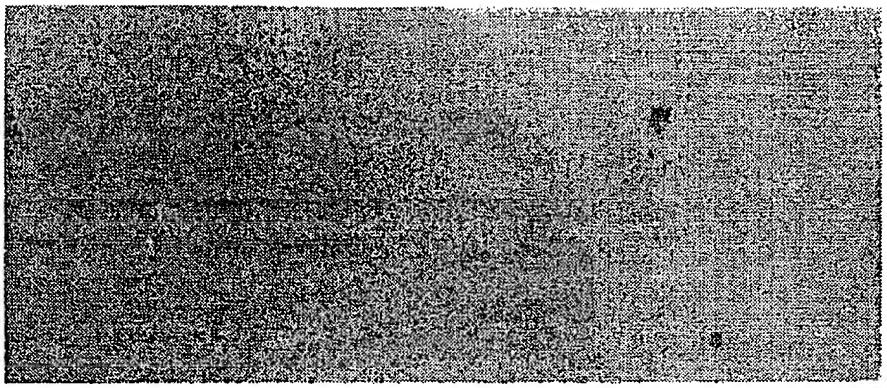


FIG. 1

```
   1  GCCCTGCTTGTAGTGTTCACCAGATCGCAGCGCAAGAACCTGTTCACTGAGATGCATGAG   60
  61  CAGCTGGGCTCTGCAGAGGCTGCGGGAGCCGAGGGGTCATGGCCAGCGCCGTCGGGCTCC  120
 121  CAGACGCCGGGTCTTGGCTGCCCTCGCCCGGCCGCCGGCGGCGACGCACCGCCTTCGCCA  180
        A  G  S  W  L  P  S  P  P  G  R  R  R  R  R  T  A  F  A  S
 181  GCCGTCACGGCAAGCGACATGGCAAGAAGTCCAGGCTGCGCTGCAGCAGAAAGCCTCTGC  240
        R  H  G  K  R  H  G  K  K  K  S  R  L  R  C  S  R  K  P  L  H
 241  ACGTGAATTTTAAGGAGTTAGGCTGGGACGACTGGATTATCGCGCCCCTAGAGTACGAGG  300
        V  N  F  K  E  L  G  W  D  D  W  I  I  A  P  L  E  Y  E  A
 301  CCTATCACTGCGAGGGCGTGTGCGACTTTCCGCTGCGCTCGCACCTTGAGCCCACTAACC  360
        Y  H  C  E  G  V  C  D  F  P  L  R  S  H  L  E  P  T  N  H
 361  ATGCCATCATTCAGACGCTGATGAACTCCATGGACCCGGGCTCCACCCCGCCTAGCTGCT  420
        A  I  I  Q  T  L  M  N  S  M  D  P  G  S  T  P  P  S  C  C
 421  GCGTTCCCACCAAACTGACTCCCATTAGCATCCTGTACATCGACGCGGGCAATAATGTAG  480
        V  P  T  K  L  T  P  I  S  I  L  Y  I  D  A  G  N  N  V  V
 481  TCTACAAGCAGTATGAGGACATGGTGGTGGAGTCCTGCGGCTGTAGGTAG  530
        Y  K  Q  Y  E  D  M  V  V  E  S  C  G  C  R  *
```

FIG. 2

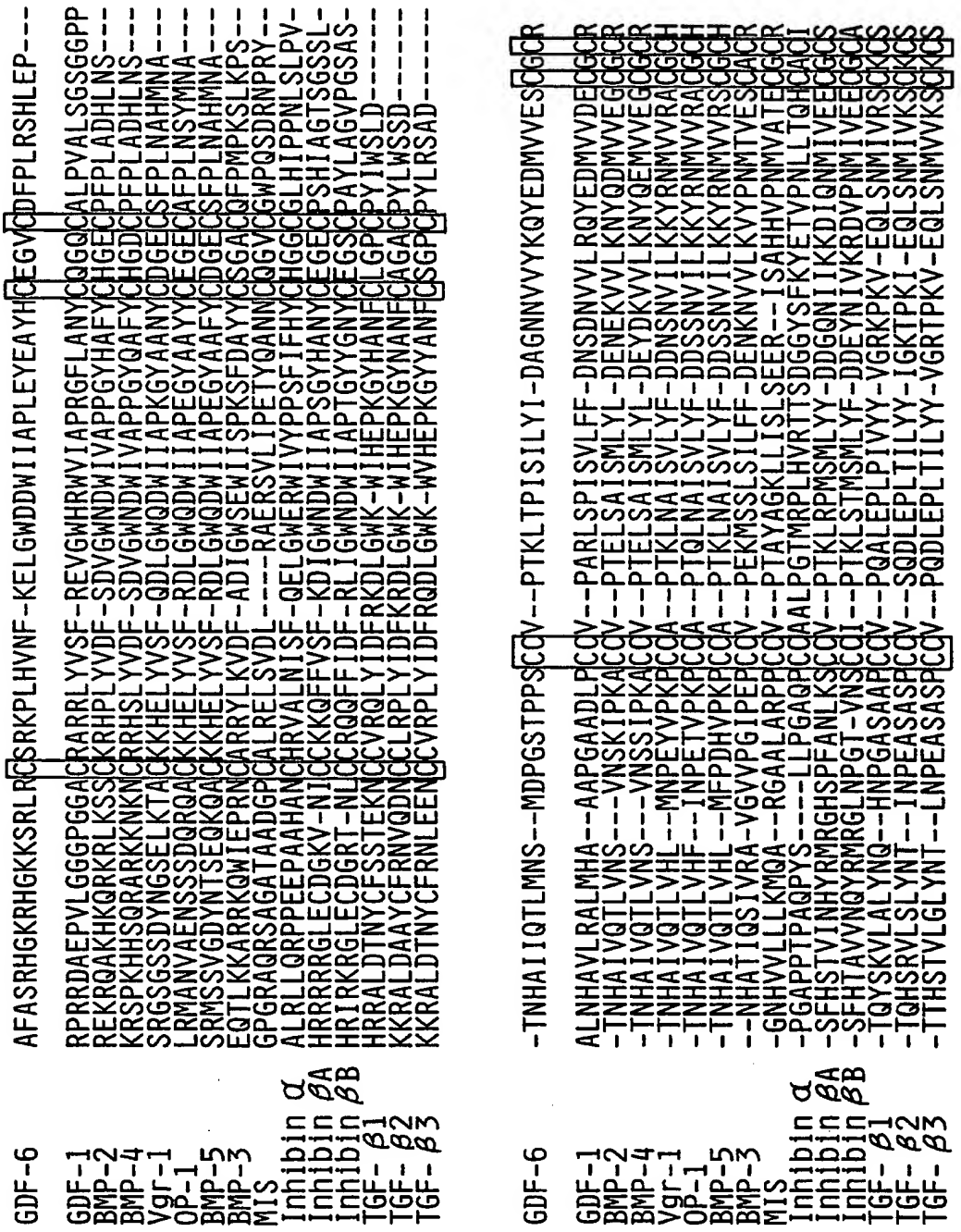
GDF-6 AFASRHGKRHGKKSRLRCSRKPLHVNF-KELGWDDWIIAPLEYEAYHCEGVCDFPLRSHLEP---
GDF-1 RPRRDAEPVLGGGPGGACRARRLYVSF-REVGWHRWVIAPRGFLANYCQGQCALPVALSGSGGPP
BMP-2 REKRQAKHKQRKRLKSSCKRHPLYVDF-SDVGWNDWIVAPPGYHAFYCHGECPFPLADHLNS---
BMP-4 KRSPKHHSQRARKKNKNCRRHSLYVDF-SDVGWNDWIVAPPGYQAFYCHGDCPFPLADHLNS---
Vgr-1 SRGSGSSDYNGSELKTACKKHELYVSF-QDLGWQDWIIAPKGYAANYCDGECSFPLNAHMNA---
OP-1 LRMANVAENSSSDQRQACKKHELYVSF-RDLGWQDWIIAPEGYAAYYCEGECAFPLNSYMNA---
BMP-5 SRMSSVGDYNTSEQKQACKKHELYVSF-RDLGWQDWIIAPEGYAAFYCDGECSFPLNAHMNA---
BMP-3 EQTLKKARRKQWIEPRNCARRYLKVDF-ADIGWSEWIISPKSFDAYYCSGACQFPMPKSLKPS--
MIS GPGRAQRSAGATAADGPCALRELSVDL----RAERSVLIPETYQANNCQGVCGWPQSDRNPRY--
Inhibin α ALRLLQRPPEEPAAHANCHRVALNISF-QELGWERWIVYPPSFIFHYCHGGCGLHIPPNLSLPV-
Inhibin βA HRRRRRGLECDGKV-NICCKKQFFVSF-KDIGWNDWIIAPSGYHANYCEGECPSHIAGTSGSSL-
Inhibin βB HRIRKRGLECDGRT-NLCCRQQFFIDF-RLIGWNDWIIAPTGYYGNYCEGSCPAYLAGVPGSAS-
TGF- β1 HRRALDTNYCFSSTEKNCCVRQLYIDFRKDLGWK-WIHEPKGYHANFCLGPCPYIWSLD------
TGF- β2 KKRALDAAYCFRNVQDNCCLRPLYIDFKRDLGWK-WIHEPKGYNANFCAGACPYLWSSD------
TGF- β3 KKRALDTNYCFRNLEENCCVRPLYIDFRQDLGWK-WVHEPKGYYANFCSGPCPYLRSAD------
GDF-6 -TNHAIIQTLMNS--MDPGSTPPSCCV--PTKLTPISILYI-DAGNNVVYKQYEDMVVESCGCR
GDF-1 ALNHAVLRALMHA--AAPGAADLPCCV--PARLSPISVLFF-DNSDNVVLRQYEDMVVDECGCR
BMP-2 -TNHAIVQTLVNS---VNSKIPKACCV--PTELSAISMLYL-DENEKVVLKNYQDMVVEGCGCR
BMP-4 -TNHAIVQTLVNS---VNSSIPKACCV--PTELSAISMLYL-DEYDKVVLKNYQEMVVEGCGCR
Vgr-1 -TNHAIVQTLVHL--MNPEYVPKPCCA--PTKLNAISVLYF-DDNSNVILKKYRNMVVRACGCH
OP-1 -TNHAIVQTLVHF--INPETVPKPCCA--PTQLNAISVLYF-DDSSNVILKKYRNMVVRACGCH
BMP-5 -TNHAIVQTLVHL--MFPDHVPKPCCA--PTKLNAISVLYF-DDSSNVILKKYRNMVVRSCGCH
BMP-3 --NHATIQSIVRA-VGVVPGIPEPCCV--PEKMSSLSILFF-DENKNVVLKVYPNMTVESCACR
MIS -GNHVVLLLKMQA--RGAALARPPCCV--PTAYAGKLLISLSEER--ISAHHVPNMVATECGCR
Inhibin α -PGAPPTPAQPYS----LLPGAQPCCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQHCACI
Inhibin βA -SFHSTVINHYRMRGHSPFANLKSCCVP--PTKLRPMSMLYY-DDGQNIIKKDIQNMIVEECGCS
Inhibin βB -SFHTAVVNQYRMRGLNPGT-VNSCCIP--PTKLSTMSMLYF-DDEYNIVKRDVPNMIVEECGCA
TGF- β1 -TQYSKVLALYNQ--HNPGASAAPCCVP--PQALEPLPIVYY-VGRKPKV-EQLSNMIVRSCKCS
TGF- β2 -TQHSRVLSLYNT--INPEASASPCCVP--SQDLEPLTILYY-IGKTPKI-EQLSNMIVKSCKCS
TGF- β3 -TTHSTVLGLYNT--LNPEASASPCCVP--PQDLEPLTILYY-VGRTPKV-EQLSNMVVKSCKCS

FIG.3

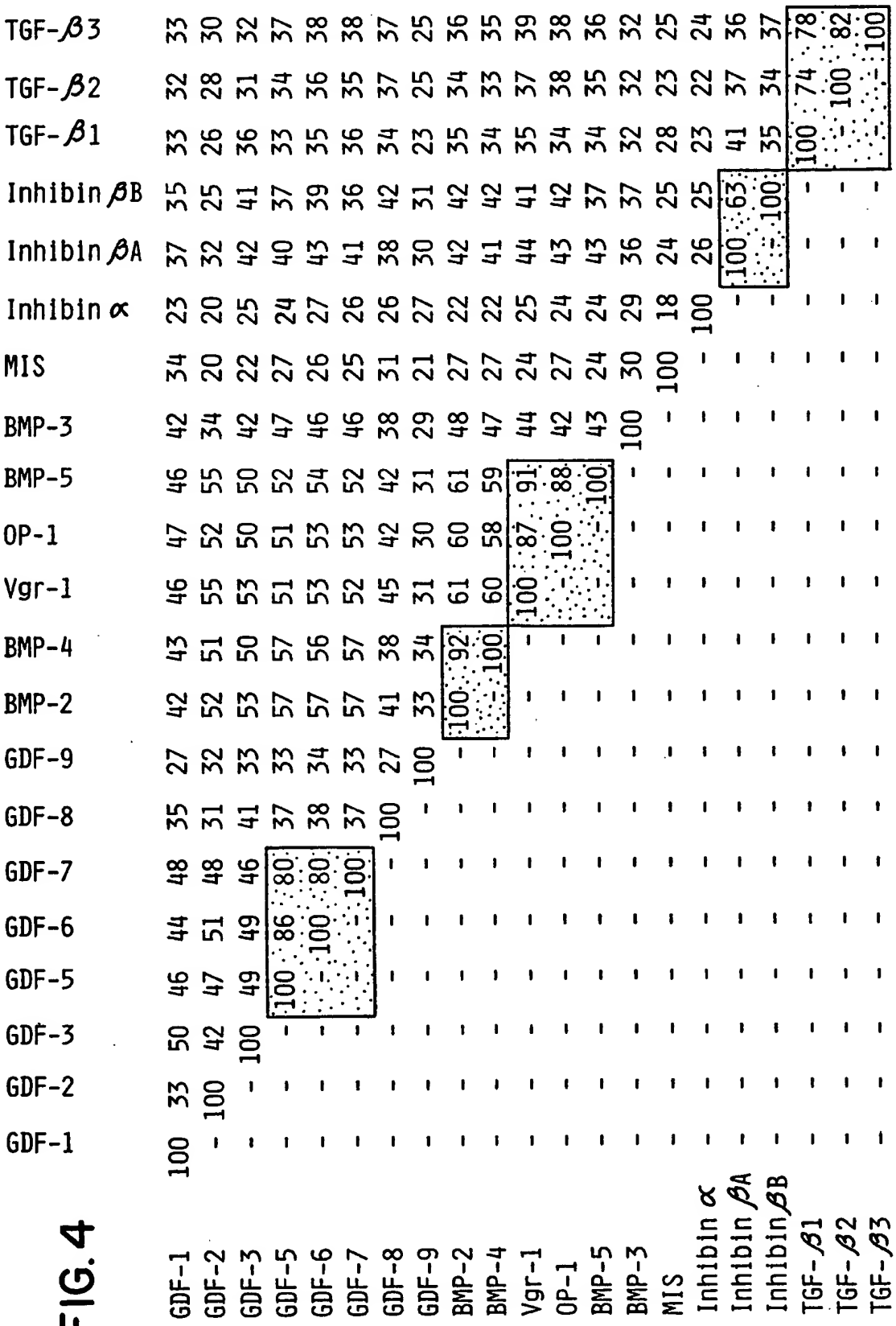
GDF-1
GDF-2
GDF-3
GDF-5
GDF-6
GDF-7
GDF-8
GDF-9
BMP-2
BMP-4
Vgr-1
OP-1
BMP-5
BMP-3
MIS
Inhibin α
Inhibin βA
Inhibin βB
TGF-β1
TGF-β2
TGF-β3

FIG. 4

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07762

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399, 397, 350; 536/23.5, 23.51; 435/320.1, 252.3, 240.1, 7.1, 7.2; 424/85.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

GENEMBL SEQUENCE DATABASES, APS, DIALOG

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A,P | WO, A, 93/16099 (NEIDHARDT ET AL) 19 August 1993, see entire document. | 1-21 |
| A,P | Nature, Volume 368, issued 14 April 1994, Storm et al, "Limb alterations in *brachypodism* mice due to mutations in a new member of the TGF ß-superfamily", pages 639-643, see entire document. | 1-21 |
| A | WO, A, 92/00382 (LEE) 09 January 1992, see entire document. | 1-21 |
| A | Proceedings of the National Academy of Sciences USA, Volume 88, issued May 1991, Lee, "Expression of growth/differentiation factor I in the nervous system: Conservation of a bicistronic structure", pages 4250-4254, see entire document. | 1-21 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>05 OCTOBER 1994 | Date of mailing of the international search report<br>20 OCT 1994 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>ELIZABETH C. KEMMERER<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07762

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | Journal of Biological Chemistry, Volume 268, Number 5, issued 15 February 1993, McPherron et al, "GDF-3 and GDF-9: Two New Members of the Transforming Growth Factor-ß Superfamily Containing a Novel Pattern of Cysteines", pages 3444-3449, see entire document. | 1-21 |
| A | Molecular Endocrinology, Volume 4, Number 7, issued 1990, Lee, "Identification of a Novel Member (GDF-1) of the Transforming Growth Factor-ß Superfamily", pages 1034-1039, see entire document. | 1-21 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07762

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 f first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-21

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/07762

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 37/24, 37/36, 39/00; C07K 3/00, 13/00, 15/00, 17/00. C07H 17/00; C12Q 1/00; G01N 33/53; C12N 5/00, 1/20, 15/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/399, 397, 350; 536/23.5, 23.51; 435/320.1, 252.3, 240.1, 7.1, 7.2; 424/85.8

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1-10, drawn to GDF-6 polypeptides, polynucleotides encoding same, vectors comprising the polynucleotides, and host cells.

II. Claims 11-21, drawn to antibodies and a diagnostic method utilizing said antibodies.

III. Claims 22, 23, and 25, drawn to a method of treating disease with an antibody.

IV. Claims 22, 24, and 25, drawn to a method of treating a disease with antisense polynucleotides.

V. Claims 22 and 25-28, drawn to a method of treating disease utilizing gene therapy techniques.

VI. Claims 29-42 drawn to targeted gene therapy techniques.

The six groups of claims listed above are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept. Specifically, group I requires polypeptides which is not required by any of groups II-VI. Similarly, group II requires antibodies which are not required by groups IV or V. Although some claims of groups III and VI require antibodies, the methods of groups III and VI require consideration of disease states and therapies which are not required by group II. Groups III through VI are drawn to separate methods, in that each method requires elements not required by the others. For instance, group III requires consideration of antibody administration, which is not required by any of the other groups. Group IV requires consideration of antisense technology, which is not required by any of the other groups. Group V requires consideration of basic gene therapy techniques which is not required by the methods of groups III or IV. Groups IV and V are separate in that group V requires consideration of liposome targeting, which is not required by any of the other groups.